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25 April, 1952.

Dear Lederberg,

I have returned from a pleasant week at Oxford to my labour of Sysiphus (research on recombination - not writing to you!) and had intended answering all your recent letters tonight. However, this morning I received your ALC telling me of Miss Cahn's inability to reproduce the S^t effect and, since my experimental notebooks are here, I thought I had better reply to this matter now. I have abstracted the relevant data from 8 experiments (+ one other involving an F+ prototroph but lacking control counts). A general account of my technique is appended at the bottom of the second sheet. These experiments deal only with 58-161/S^t X W677 (&W677/S^r) matings and are, I think, a reasonable sample of my total. While, as you will see, the A/S^t:A ratio of prototrophs is very variable and the A/S^t prototroph count always markedly lower than that given by A, I have never failed to obtain prototrophs with A/S^t suspensions. Against this, I have never succeeded, in a lesser but considerable no. of expts. under the same conditions, in obtaining a single prototroph colony from matings involving W677/S^t, despite a high survival rate in some expts. and although all matings were on min. agar + B_1 (no SM) while the majority of 58-161/S^t matings were on MA + B_1 + SM200, thus continuing the SM action. I was (and am) quite sure of the results themselves and considered that they justified a preliminary communication to "Nature". As you say, the unequivocal results of crossing expts. on MA + SM, as well as the association of fertile S^t with F+, also strongly support the results. Nevertheless I am very interested in your views and Miss Cahn's results. The latter I cannot understand at the moment and can only suggest that she tries again. You have made a most important and valid point about the possible effect of cytoplasmic SM in S^t cells in inhibiting development of an S^s ♂-gamete as, of course, this would only become a significant factor in F-/S^t X F+/S^r mates. This had not occurred to me. It is a very neat point & one to which, at the moment, I can only reply that I <u>have</u> obtained prototrophs with A/S^t X B matings (using 1000 ug./ml. SM) though with less efficiency than with B/S^r (vide Expt. of 5.10.51, p.2). I will see if I can obtain some SM-antagonist from Mill Hill. If I can I will send you some. I had considered the possibility that the drop in recombination rate using A/S^t suspensions might be due to deleterious action of adsorbed SM (? due to charge) on the penetrability or other function of a "gamete" and had thought of trying the anti-SM effect of hydroxylamine but decided it was'nt worth the trouble on account of its cytotoxicity.

The fact is that my results with SM (whatever their true interpretation) and UV (do'nt forget this!), together with the stimulus of correspondence with you and Cavalli, opened up such an interesting field that I preferred to rush on with the tanks while the going was good and have not yet attempted to consolidate my rear. I have no infantry and must go back and do it myself when the front stabilises a bit! This applies especially to the S^t work which so obviously requires definition. In the consolidation programme which I have provisionally planned when I can get down to it I have noted the following points:

1. Attempts to increase the effectiveness of SM in sterilisation by addition of sulphonamide or penicillin.
2. Analysis of the fertility of S^t suspensions in terms of physiological conditions of the cells and after UV enhancement. The few experiments I have done strongly suggest that A/UV cultures subsequently SM-treated show a marked increase in fertility, though my technique h[illegible] differed from that

used in the previous S^t expts. I think I told you before that washings of agar suspensions in saline show an extremely low recombination rate. If, however, the suspensions are suspended in broth at 37°, the rate rises rapidly to a maximum in about an hour. This applies to 58-161 and W677, although the UV effect is restricted to 58-161 alone. This same phenomenon also occurs with even young broth cultures but to a much lesser extent. It is clearly distinct from the UV effect & may be of importance, especially when coupled with morphological study. Results of Expts. 1, 2 & 3 also suggest that, under properly controlled conditions, agar suspensions incubated in broth and subsequently treated with SM may prove better material for studying the S^t phenomenon than young broth cultures. Incidentally, this fertility enhancement resulting from suspending agar growths in broth is not associated with increased liberation of lambda as is the UV effect.

3. Study of the effect of UV on non-lysogenic K-12 mutants, which Lwoff has promised to provide.

As regards the techniques I have used in the S^t expts., I began by using 24 hr. agar washings suspended in NB + SM to a final conc. of 1000 µg./ml. for 2 hrs. at 37°. This appeared to give good prototroph counts but "sterility" was poor. I therefore aimed at producing sterility (as judged by no growth from the S^t inoculum X 2 on NA after 48-72 hrs. at 37°) & found that this was best achieved by [redacted] the regimen used in expts. 5, 6, 7, 8. The intermediate log phase culture was designed to eliminate "persisters" (temporarily dormant cells from the overnight culture) I thought that if the 2nd. log phase culture was grown for more than 4 hrs. before adding SM, allowing for another division or so some cells might have entered the stationary phase and become more resistant to SM. You will observe that this regimen, while quite effective in producing "sterility", appeared (usually but not always) to have a markedly adverse effect on the fertility of S^t suspensions. This may be due (as you wish to imply, I guess) to the fact that most of the cells were now really sterilised. On the other hand the shaking may have promoted alteration of $F+ \rightarrow F-$ phenocopy, which I could interpret as a relative failure of "gamete" extrusion (is this good 'sciencemanship'?). An alternative explanation which I previously entertained to account for the low fertility of S^t suspensions was this. When untreated heterologous mutant suspensions are mixed and spread on minimal agar a certain amount of growth (& physiological function) goes on. Thus, living cells constitute a dynamic population so that cells which had not extruded their genes ("gamete") on first mixing could still do so. On the other hand SM-treatment "freezes" the population so that only those cells which had exposed their "gametes" at the time of initiation of treatment would be effective in recombination.

I feel that this problem of S^t suspensions is so intimately associated with other more recent findings that it (& certainly its interpretation) cannot be separated from the problem as a whole and its solution. I therefore propose, in my paper which will be published in the same issue of the JGM as yours & Cavalli's, to stick to my theory. This has served me well in prediction & I feel that it may well present a facet of the truth. Moreover, I feel that the most productive results usually arise from two or more persons attacking a problem from different perspectives. Even if my views are completely wrong, as they may be, they will have served a useful purpose in being provocative. As regards the morphological approach, as you know Mrs. Klieneberger-Nobel is working on this in London & I am helping her as much as I can. I have supplied her with F+ & F- strains of 58-161 & W677 which may prove useful in separating morphological distinctions associated with the sex process from those due to nutritional factors.

[redacted]

Within the next two days I will send you a copy of my paper to the SGM & this will give you, I hope, a lucid account of my theory. I agree with you about semantics and would like to point out that, in this paper, I have used the word "transformation" in its common connotation and with no esoteric implications. The word "transduction" does have rather a specific meaning and, since time precluded any semantic discussion, I picked on "transformation" to express the F+—F- alteration. I am sorry this paper has been so long but I have been held up by the photographic dept. Thanks for your remarks about the persistent diploids, which I think I understand, & about the possibility of the segmental eliminations. I am not qualified to criticise or discuss such views from you or Cavalli but it does strike me that fitting the facts into an orthodox genetical picture is involving increasing complications and that Occam's razor may prove a double-edged weapon! I think we both have open minds about this problem and to attempt to describe and explain what we observe in terms of a context we already know and understand is not a failing but inevitable. Incidentally I have told Cavalli that I have no desire to establish any priority (which I do not have in fact) over the F+ business and, therefore, do not intend to publish an abstract of my SGM talk in the next number of the journal. Your "Genetics" paper will, I hope, come out first and then our synchronous publications in the JGM.

Yours sincerely,

William Hayes

Mating experiments with 5^{or} cultures.

A = 5-8-161.
B = W677.

Date of Expt.	PROTOTROPH COUNTS (Av. of duplicate, triplicate & subtriplicate assays).		TOTAL COUNTS (48-72 hrs. agar).		DURATION SM ACTION. (hours).	AGITATION.	GROWTH CONDITIONS OF CULTURE TREATED. (before SM).
1.3.5-1.	A × B/5^{r}	11	A(10^{-6})	153			
	A/5^{r} × B/5^{r}	2	B(10^{-6})	134	2	—	24 hr. agar-suspended in broth + SM.
(1)			A/5^{r}(10^{-3})	0			
5-3.5-1.	A × B/5^{r}	5-	A (10^{-6})	298			
	A(10^{-2}) × B/5^{r}	0	B/5^{r}(10^{-6})	369	2	—	"
	A/5^{r} × B/5^{r}	7	A/5^{r}(10^{-2})	134			
(2)			A/5^{r}(10^{-3})	36			
7.3.5-1.	A × B/5^{r}	49	A (10^{-6})	353			
	A(10^{-1}) × B/5^{r}	7	B/5^{r}(10^{-6})	520			
	A(10^{-2}) × B/5^{r}	0	A/5^{r}(10^{-1})	100	5-	—	"
(3)	A/5^{r} × B/5^{r}	8	A/5^{r}(10^{-2})	2.			
20.4.5-1.	A × B/5^{r}	112	A(10^{-6})	270			
	A(10^{-1}) × B/5^{r}	6	B/5^{r}(10^{-6})	224	18 1/2.	—	4 hr. broth.
	A(10^{-2}) × B/5^{r}	2	A/5^{r}(10^{-2})	350			
(4)	A/5^{r} × B/5^{r}	19					
8.5-5-1.	A × B/5^{r}	65-	A(10^{-6})	176			
	A(10^{-1}) × B/5^{r}	6	B/5^{r}(10^{-6})	124	※a. 18	—	
	A(10^{-2}) × B/5^{r}	1.5-	※A/5^{r}(a)(10^{-1})	229			
	A(10^{-3}) × B/5^{r}	0.5-	" (a) (10^{-2})	25-			4 hr. broth.
(5)	※A/5^{r}(a) × B/5^{r}	1	" (a) (10^{-3})	0	※b. 18	+	
	※A/5^{r}(b) × B/5^{r}	8	※A/5^{r}(b)(10^{0})	(0)			
20.9.5-1.	(A × B/5^{r})/5-	331	A(10^{-6})	162			
	A(10^{-3}) × B/5^{r}	3	B/5^{r}(10^{-6})	78	4	+	2 1/2 hr. broth.
(6)	A/5^{r} × B/5^{r}	8	A/5^{r}(10^{0})	(0)			
27.9.5-1.	(A + B/5^{r})/10.	36	A (10^{-6})	147			
	A(10^{-3}) × B/5^{r}	1	B/5^{r}(10^{-6})	153-	4	+	2 3/4 hr. broth.
(7)	A (10^{-4}) × B/5^{r}	0	A/5^{r}(10^{0})	(0)			
	A/5^{r} × B/5^{r}	11					

Date.	PROTOTROPH COUNTS.		TOTAL COUNTS.		DURATION SM ACTION	AGITATION	GROWTH CONDITIONS.
5.10.51.	$(A \times B/S^r)/10.$	120	$A(10^{-6})$	388			
	$(A/S^r \times B)/10.$	60	$A/S^r(10^{-6})$	178			
	$(A \times B)/10.$	86	$B(10^{-6})$	349			
	$A(10^{-4}) \times B/S^r$	1	$B/S^r(10^{-6})$	163	4	+	3½hr. broth.
(8)	$A/S^r \times B(10^{-4})$.	0	$A/\underline{S}^t(10^0)$	66			
	$A/\underline{S}^t \times B/S^r$	414	$B/\underline{S}^t(10^0)$	9			
	$A/\underline{S}^t \times B.$	208					
	$B/\underline{S}^t \times A/S^r$	0					
	$B/\underline{S}^t \times A.$	0					
27.2.52.	$P = B_1^-, S^s F^+$ prototroph ex (A×B) mating.						
	$A \times B/S^r$	36	—				
	$A/\underline{S}^t \times B/S^r$	27	—		4	—	3½hr. broth.
	$P \times B/S^r$	12	—				
	$P/\underline{S}^t \times B/S^r$	6					

METHODS.

SM-treatment - Expts.1,2,3. Overnight nutrient agar(NA)culture suspended in small vol.saline → diluted in X 20 vol.nutrient broth at 37°(NB37) containing SM1000µg./ml. → 37° 2-5 hrs. → washed X 3 & resuspended in appropriate small vol.saline-buffer.

Expts.5,6,7,8. 0.1 ml.overnight NB culture(ex Dorset egg at 4°C.) → 5 ml. NB37 → mechanically shaken 37° 2 hrs. → 0.5-1.0 ml. → 50 ml. NB37 → 2-4 hrs.37° → SM added to 1000(rarely 2000)µg./ml. → shaken 37° 4-18 hrs. → wash X 3 as before.

(The reason for all this is hidden in the letter).

Control untreated suspensions were always an aliquot of the same NA suspension or NB culture, identically treated except for refrigeration at 4° instead of SM-treatment.

Heterologous mating suspensions were prepared in an identical way to the control untreated suspensions.

Control Counts. Immediately before mating, appropriate dilutions of each suspension were made & one standard loop vol.(0.0055 ml.) spread on NA(in duplicate or triplicate). Counts of S^t suspensions were made after 48-72 hrs. at 37°.

Mating. Equal vols.suspension mixed & one standard loop vol.spread on surface of min.agar(+ B_1:with or without SM200 according to expt.)in (usually)small cm.diam.plates(duplicate, triplicate or quadruplicate).

Total counts quoted = (No.organisms of each suspension in mixture actually plated on min.agar) X 2. Prototroph counts usually read 40-48 hrs.37°.